PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING DOCUMENT TRANSMITTED

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as designated Office

Date of mailing (day/month/year)	
26 May 1999 (26.05.99)	
International application No.	**International filing date** (day/month/year)
PCT/GB97/01248	08 May 1997 (08.05.97)
Applicant	
APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al	

The International Bureau transmits herewith the following documents and number thereof:

_____ cop(ies) of priority document(s) (Rule 17.2(a))

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

H. Zhou

Telephone No.: (41-22) 338.83.38

09/180335

PATENT COOPERATION TREATY

1743

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

WOODMAN, Derek
Frank B. Dehn & Co.
179 Queen Victoria Street
London EC4V 4EL
ROYAUME-UNI

Date of mailing (day/month/year) 26 May 1999 (26.05.99)	
Applicant's or agent's file reference 40.10.63571/001	**IMPORTANT NOTIFICATION**
International application No. PCT/GB97/01248	International filing date (day/month/year) 08 May 1997 (08.05.97)
International publication date (day/month/year) 20 November 1997 (20.11.97)	Priority date (day/month/year) 09 May 1996 (09.05.96)
Applicant APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
09 May 1996 (09.05.96)	9609653.2	GB	21 May 1999 (21.05.99)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

H. Zhou

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 December 1997 (15.12.97)	
International application No. PCT/GB97/01248	**Applicant's or agent's file reference** 40.10.63571/001
International filing date (day/month/year) 08 May 1997 (08.05.97)	**Priority date** (day/month/year) 09 May 1996 (09.05.96)
Applicant COOKSON, Alan, Derek et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

01 December 1997 (01.12.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer H. Zhou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

WOODMAN, DEREK
FRANK B. DEHN & CO.
179 Queen Victoria Street
++++++++++++++++++++++++++++++++
London EC4V 4EL
GRANDE BRETAGNE

FILE 63571/001
16 FEB 1998

PCT

WRITTEN OPINION

(PCT Rule 66)

GA

Date of mailing *(day/month/year)*	16. 02. 98
Applicant's or agent's file reference 40.10.63571/001	**REPLY DUE** within 3 month(s) from the above date of mailing

International application no.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/01248	08/05/1997	09/05/1996

International Patent Classification (IPC) or both national classification and IPC
G01N33/543

Applicant
APPLIED RESEARCH SYSTEMS ARS HOLDING NV et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and / or arguments, see Rule 66.4bis.
For an informal communication with the examiner, see Rule 66.6.

DUE DATES NOTED
16/5/98

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 09/09/1998

Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. (+49-89) 2399-0. Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer / Examiner Goetz, M Formalities officer (incl. extension of time limits) Houyez-Stevens, M Telephone No. (+49-89) 2399-8163

Form PCT/IPEA/408 (Cover sheet) (January 1994)

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-15 as originally filed

Claims, No.:

1-15 as originally filed

Drawings, sheets:

1-2 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1 - 5, 7 - 10, 12
Inventive step (IS)	Claims	1 - 15
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

BASIS OF THE REPORT

The examination is being carried out on the **following application documents**:

Description, pages:

1-15 as originally filed

Claims, No.:

1-15 as originally filed

Drawings, sheets:

1-2 as originally filed

STATEMENT

1. Examples 1 and 4 of

D1 = EP-A-0 184 600

(pages 18 and 21) describe the determination of the Hb content of a sample by binding Hb to anti-Hb antibodies coated on the surface of glass waveguides and directly and continuously measuring the optical absorption at 410 nm over a given time period. In a first step, standard curves are established for known Hb concentrations (see. Fig. 6), which can be used for the determination of the Hb content in unknown samples.

Example 6 of **D1** (page 24) describes the simultaneous determination of human IgG and HSA in a sample by binding fluorescently labelled antibodies to antigen bound on capture antibodies immobilized on the surface of glass waveguides, and directly and continuously measuring the emitted fluorescent light at 510 nm over a given time period. In a first step, standard curves are established for known antigen concentrations (see. Fig. 9), which then are used for the determination of the IgG or HSA content in unknown samples.

In the said examples, the glass waveguides are designed to constitute a sample containing compartment (see Figs. 2 and 4a/4b).

The methods according to **D1** encompass the subject-matter of present claims 1 - 5 and 7 - 10 which do not therefore meet the requirements pursuant to Art. 33(2) PCT.

2. In any of examples 1, 4 and 6 of **D1**, the assay system is calibrated by establishing standard curves for samples of known analyte concentration.

Hence, claim 12 does not meet the requirements according to Art. 33(2) PCT either.

It appears that claim 12 seeks protection for a most trivial technique known by any skilled person, namely the build-up of standard curves using reference samples, where several readings for a given parameter P_x are taken at different times $T = t_0, t_1, ..., t_n$ and correlated with the known concentrations $C = c_1, ..., c_n$.

3. Another assay which uses solid waveguide bound antibodies to measure over a predetermined time interval the fluorescence emitted by secondary antibody labelled antigen in a direct and continuous manner, whereby standard curves are established using known antigen concentrations and mathematical recursion methods, is disclosed in

D2 = EP-A-0 667 528,

see column 4/line 32 - column 5/line 31, column 7/lines 29 - 34, column 9/lines 7 - 17, column 9/line 51 - column 10/line 1.

Hence, also in the light of **D2**, claims 1 - 5 and 8 - 10 would not appear to meet the requirements pursuant to Art. 33(2) PCT.

4. The subject-matter of claims 6 and 11 relates to a commonly known technical embodiment (see e.g. the capillary fill devices of **D3 = WO90/14590**) and does not involve an inventive step. The said claims do not therefore meet the requirements pursuant to Art. 33(3) PCT.

5. Assay devices comprising bar code strips to store machine readable data as well as their incorporation into test kits are known in the state of the art, see **D4 = WO92/14136**, page 18/lines 6 - 10 and page 19/line 35 - page 20/line 2, see also **D5 = US-A-5 120 662**, column 7/lines 41 - 62 and Fig. 11.

   Storing calibration data on such bar code strips does not therefore appear to involve an inventive step. Claims 13 - 15, directed to such subject-matter, do not therefore meet the requirements pursuant to Art. 33(3) PCT.

CERTAIN DEFECTS

According to Rule 5.1 (a)(ii) PCT, the teaching provided by documents **D1** and **D2** should briefly be discussed in the description.

EPA/EPO/OEB
D-80298 München
(089) 2399-0
TX 523 656 epmu d
FAX (089) 2399-4465

Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

WOODMAN, DEREK
FRANK B. DEHN & CO.
179 Queen Victoria Street
London EC4V 4EL
GRANDE BRETAGNE

FILE 63571/03
- 3 JUL 1998
RECEIVED
ANSD 6A

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)*: 01. 07. 98
Applicant's or agent's file reference: 40.10.63571/001	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/01248	08/05/1997	09/05/1996

Applicant: APPLIED RESEARCH SYSTEMS ARS HOLDING NV et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer
Hebert, W.
Tel. (+49-89) 2399-8161

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
40.10.63571/001		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/01248	08/05/1997	09/05/1996
International Patent Classification (IPC) or national classification and IPC		
G01N33/543		
Applicant		
APPLIED RESEARCH SYSTEMS ARS HOLDING NV et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01/12/1997	01. 07. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0. Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Goetz, M Telephone No. (+49-89) 2399-8697

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/01248

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1,2,4,6,7,10-15	as originally filed			
3,3a,5,8,9	as received on	15/06/1998	with letter of	11/06/1998

Claims, No.:

1-11	as received on	15/06/1998	with letter of	11/06/1998

Drawings, sheets:

1,2	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1 - 11
	No:	Claims
Inventive step (IS)	Yes:	Claims 9 -11
	No:	Claims 1 - 8
Industrial applicability (IA)	Yes:	Claims 1 - 11
	No:	Claims

2. Citations and explanations

see separate sheet

Re Item I
Basis of the report

The examination is being carried out on the following application documents:

Description, pages:

1,2,4,6,7,10-15	as originally filed			
3,3a,5,8,9	as received on	15/06/1998	with letter of	11/06/1998

Claims, No.:

1-11	as received on	15/06/1998	with letter of	11/06/1998

Drawings, sheets:

1,2	as originally filed

Re Item V
Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. None of the available documents cited in the International Preliminary Search Report discloses the subject-matter according to any of claims 1 - 11, which subject-matter is therefore novel.

   Claims 1 - 11 therefore meet the requirements pursuant to Art. 33(2) PCT.

2. The principle underlying the presently claimed subject-matter, i.e. the use of kinetic measurements in the quantitative determination of an analyte, wherein a component of the assay system becomes at least partly bound to a solid phase, is disclosed in **EP-A-0 667 528,** relating to an assay which uses solid waveguide bound antibodies to measure over a predetermined time interval the fluorescence emitted by secondary antibody labelled antigen in a **direct and continuous** manner, whereby standard curves are established using known antigen concentrations and mathematical recursion methods (see column 4/line 32 - column 5/line 31, column 7/lines 29 - 34, column 9/lines 7 - 17, column 9/line 51 - column 10/line 1).

The findings as set forth on page 3/lines 23 - 30 of the present description, would appear to have been suggested in **EP-A-0 667 528**, using a somewhat more sophisticated language, in column 6/line 36 - column 7/line 34.

The feasibility of kinetic measurements in immunoassays being basically known, the subject-matter of claims 1 - 7 does not appear to involve an inventive step and does not therefore comply with Art. 33(3) PCT.

3. Claim 8 seeks protection for a trivial technique known by the skilled person, namely the build-up of standard curves using reference samples having known analyte concentrations $C = c_1, ..., c_a$, where several readings for a given parameter P_x are taken for each reference sample at a plurality of times $T = t_0, t_1, ..., t_n$ and correlated with the known concentrations $C = c_1, ..., c_a$, wherein the number n of readings within a given time interval is sufficiently high to make the determination a continuous one (e.g. by making readings in intervals of 5 seconds, as mentioned in the present description).

   Claim 8 cannot therefore be considered to meet the requirements according to Art. 33(3) PCT.

4. The subject-matter of claims 9 - 11 is not suggested by the available documents cited in the International Preliminary Search Report. The said claims therefore comply with Art. 33(3) PCT.

the assay must be allowed to attain an arbitrarily determined equilibrium at which point a single end-point measurement of the signal is made. The speed with which equilibrium is reached may be prohibitively slow and this in itself can introduce errors in the measured rate of change of signal which will be critically dependent on the prevailing conditions (eg. temperature, viscosity). Clearly it is not possible in such a system to obtain quick and accurate measurements of the ligand concentration.

Kinetic measurements have also been used to determine the concentration of an unknown in some immunoassays, as disclosed in EP-A-667528 (Daikin Industries, Limited). However, such assays do not involve the continuous monitoring of the concentration of the unknown.

In other assays systems, for example that disclosed in EP-A-184600 (Battelle Memorial Institute) kinetic measurements may be made, but are not used to determine the concentration of an unknown in a sample, with instead a single final measurement being used in this regard.

The present invention is based on the finding that, in assay methods, during the course of which a component of the assay system becomes directly or indirectly bound to, or adsorbed on, the surface of a solid body, a reliable measurement of said bound or adsorbed component (ie. without interference from the free component in solution) can be obtained by direct and continuous monitoring of said component.

It should be emphasised that the method of the invention relates to assay systems of both the direct and indirect variety, the only requirement being that they involve the binding of a component of the assay system to the surface of a solid body. Direct assay methods may typically involve monitoring the reflected and/or generated signal within an irradiated solid optical structure (eg. a waveguide) in order to

determine the extent to which (or the rate at which) the optical characteristics of said optical structure and/or the generated signal are altered by the biochemical complexation of a ligand and specific binding partner which is bound to said optical structure (eg. antigen/antibody complexation). Indirect assay methods may typically involve monitoring a label (eg. a fluorophore) bound to one or more of the components present in the assay and directly or indirectly to the solid body. Such methods are described for example in

determine an unknown sample. In one embodiment the analyte dependent parameter is an analyte dependent optical parameter (i.e. a measurable optical property or effect) but parameters relating to electrochemical or piezoelectric properties/effects may be used.

In a further embodiment, the use or method according to the invention may be applied to an analyte of known concentration for the purposes of calibration.

In a particular preferred embodiment, said method comprises the steps of:

(a) calibrating the assay system for x samples each of known analyte concentration (C_a) by measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z),

(b) for an analyte of unknown concentration (C_b) measuring continuously n independent values of an analyte-dependent parameter (P_d) each at time t_e after the onset of incubation,

(c) combining the data (P_d, t_e) from step (b) with the calibration data (P_z, t_y, C_a) from step (a) to calculate the unknown dose of analyte (C_b) at time t_e.

The analyte-dependent parameter referred to above may conveniently be any parameter associated with the interaction between applied radiation and the relevant bound assay component and includes but is not limited to light-absorbing, scattering, fluorescence emission, phosphorescence emission, luminescence emission (including chemiluminescence, bioluminescence and electrochemiluminescence) or colour emission properties. The term is also intended to encompass the measurable effects which the bound component may have for example on the refractive index or transmittability of the optical surface, on total internal reflection or surface plasmon resonance (SPR) within the solid optical body, or interactions with evanescent waves at the surface of the body. Devices and techniques for measuring such analyte dependent optical parameters or manipulating the

of the invention. Such operator determined variables include the number of standards (referred to as x above) which might typically be three or more, the number of devices, the number of readings, the time interval between readings and the total period over which calibration is carried out.

After filling a device with a particular standard, measurements of the analyte dependent parameter (referred to more generally as P_z above) are taken at regular intervals, such as every five seconds, for as long as is appropriate. This procedure is optionally repeated for further devices, yielding response data at each time point (referred to as t_y above) for all of the standard analytes. For each time point t_y it is, therefore, possible to produce a standard curve of P_z vs C_a (appropriate to time t_y). In one embodiment, the (P_z, C_a) data may be fitted to a standard equation such as an n parameter logistic equation, or appropriate algorithm, using any conventional fitting method such as a least squares method.

In step (b) of the method according to the invention, the unknown analyte-dependent parameter (referred to as P_d above) may be measured at any time-point (referred to as t_e above) and used to determine a concentration by interpolation from the standard curve (P_z, C_a) for that time point. Appropriate smoothing software may be used to improve the accuracy of the estimation of concentration of analyte in the sample. Typically the (t_e, C_b) data obtained in this step are manipulated to give a dose versus time profile for the sample, an example of which is given in Figure 2.

As has previously been emphasised, the method according to the invention is a kinetic method and in step (b) the interval between readings is operator determined and is typically of the order of less than 60s, particularly less than 30s, especially less than 10s and more especially 5s or less.

In practice, it is envisaged that the calibration

data from step (a) of the method according to the invention may be prepared by a manufacturer for each batch of reagents and presented as a series of standard kinetic curves. These curves would then be supplied to the customer via convenient means for storing machine readable encoded data such as software, bar codes or magnetic strips for each batch of reagents. Thus, for example, on running unknown samples, the appropriate instrument would carry the calibration curves in its software and use them as "look up tables" in order to calculate the dose of analyte in the sample under test.

Thus in a further aspect the present invention provides a method of calibrating an assay system comprising step (a) as hereinbefore defined and optionally thereafter fitting the (P_z, C_a) data to a standard equation (appropriate to time t_y). A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_z, t_y, C_a as hereinbefore defined and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown analyte forms a further aspect of the invention.

One technological area which has undergone significant advancement in recent years is the so-called point-of-care assay systems. These rely on very accurate, sensitive and rapid methods of assay to enable successful near patient testing to be performed. Clearly therefore the present invention, with the advantages referred to hereinbefore, lends itself to such technology.

The method of the invention is particularly applicable to assays of antigens or antibodies, i.e. to immunoassays, and in one embodiment of the invention the ligand under assay is an antigen and the specific binding partner comprises an antibody to the said antigen. However, as mentioned above, the invention is not to be taken as limited to assays of antibodies or antigens. Examples of ligands which may be assayed by

Claims

1. A method of assay in which a component becomes at least partly bound to a solid body characterised in that an analyte dependent parameter associated with said component is measured in a direct and continuous manner and in that said measured analyte dependent parameter is manipulated to quantitatively determine an unknown sample and in that the results of the determination are monitored continuously.

2. A method as claimed in claim 1 wherein said solid body is an optical waveguide.

3. A method as claimed in either claim 1 or claim 2 wherein said analyte dependent parameter is an optical parameter.

4. A method as claimed in any of claims 1 to 3 wherein said optical parameter is fluorescence emission.

5. A method as claimed in any preceding claim wherein said solid body is in the form of a sample containment device.

6. A method as claimed in claim 5 wherein said device is a capillary fill device.

7. A method as claimed in any preceding claim comprising the steps of

(a) calibrating the assay system for x samples each of known analyte concentration (C_a) by measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z),

(b) for an analyte of unknown concentration (C_b) measuring continuously n independent values of an analyte-dependent parameter (P_d) each at time t_s after

the onset of incubation,

(c) combining the data (P_d, t_e) from step (b) with the calibration data (P_z, t_y, C_a) from step (a) to calculate the unknown dose of analyte (C_b) at time t_e.

8. A method of calibrating an assay system for x samples each of known analyte concentration (C_a) comprising:

(a) measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z); and, optionally

(b) fitting the calibration data to a standard equation.

9. A method as claimed in claim 8 further comprising the step of storing said calibration data on a means for storing machine readable encoded data.

10. A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_z, C_a, t_y as defined in claim 7 and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown analyte.

11. A kit as claimed in claim 10 characterised in that the data storing means comprises a bar code marked on the device.

the assay must be allowed to attain an arbitrarily determined equilibrium at which point a single end-point measurement of the signal is made. The speed with which equilibrium is reached may be prohibitively slow and this in itself can introduce errors in the measured rate of change of signal which will be critically dependent on the prevailing conditions (eg. temperature, viscosity). Clearly it is not possible in such a system to obtain quick and accurate measurements of the ligand concentration.

The present invention is based on the finding that, in assay methods, during the course of which a component of the assay system becomes directly or indirectly bound to, or adsorbed on, the surface of a solid body, a reliable measurement of said bound or adsorbed component (ie. without interference from the free component in solution) can be obtained by direct and continuous monitoring of said component.

It should be emphasised that the method of the invention relates to assay systems of both the direct and indirect variety, the only requirement being that they involve the binding of a component of the assay system to the surface of a solid body. Direct assay methods may typically involve monitoring the reflected and/or generated signal within an irradiated solid optical structure (eg. a waveguide) in order to determine the extent to which (or the rate at which) the optical characteristics of said optical structure and/or the generated signal are altered by the biochemical complexation of a ligand and specific binding partner which is bound to said optical structure (eg. antigen/antibody complexation). Indirect assay methods may typically involve monitoring a label (eg. a fluorophore) bound to one or more of the components present in the assay and directly or indirectly to the solid body. Such methods are described for example in

determine an unknown sample. In one embodiment the analyte dependent parameter is an analyte dependent optical parameter (i.e. a measurable optical property or effect) but parameters relating to electrochemical or piezoelectric properties/effects may be used.

In a further embodiment, the use or method according to the invention may be applied to an analyte of known concentration for the purposes of calibration.

In a particular preferred embodiment, said method comprises the steps of:

(a) calibrating the assay system for x samples each of known analyte concentration (C_x) by measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x),

(b) for an analyte of unknown concentration (C_n) measuring continuously n independent values of an analyte-dependent parameter (P_n) each at time t_n after the onset of incubation,

(c) combining the data (P_n, t_n) from step (b) with the calibration data (P_x, t_x, C_x) from step (a) to calculate the unknown dose of analyte (C_n) at time t_n.

The analyte-dependent parameter referred to above may conveniently be any parameter associated with the interaction between applied radiation and the relevant bound assay component and includes but is not limited to light-absorbing, scattering, fluorescence emission, phosphorescence emission, luminescence emission (including chemiluminescence, bioluminescence and electrochemiluminescence) or colour emission properties. The term is also intended to encompass the measurable effects which the bound component may have for example on the refractive index or transmittability of the optical surface, on total internal reflection or surface plasmon resonance (SPR) within the solid optical body, or interactions with evanescent waves at the surface of the body. Devices and techniques for measuring such analyte dependent optical parameters or manipulating the

of the invention. Such operator determined variables include the number of standards (referred to as x above) which might typically be three or more, the number of devices, the number of readings, the time interval between readings and the total period over which calibration is carried out.

After filling a device with a particular standard, measurements of the analyte dependent parameter (referred to more generally as P_x above) are taken at regular intervals, such as every five seconds, for as long as is appropriate. This procedure is optionally repeated for further devices, yielding response data at each time point (referred to as t_x above) for all of the standard analytes. For each time point t_x it is, therefore, possible to produce a standard curve of P_x vs C_x (appropriate to time t_x). In one embodiment, the (P_x, C_x) data may be fitted to a standard equation such as an n parameter logistic equation, or appropriate algorithm, using any conventional fitting method such as a least squares method.

In step (b) of the method according to the invention, the unknown analyte-dependent parameter (referred to as P_n above) may be measured at any time-point (referred to as t_n above) and used to determine a concentration by interpolation from the standard curve (P_x, C_x) for that time point. Appropriate smoothing software may be used to improve the accuracy of the estimation of concentration of analyte in the sample. Typically the (t_n, C_n) data obtained in this step are manipulated to give a dose versus time profile for the sample, an example of which is given in Figure 2.

As has previously been emphasised, the method according to the invention is a kinetic method and in step (b) the interval between readings is operator determined and is typically of the order of less than 60s, particularly less than 30s, especially less than 10s and more especially 5s or less.

In practice, it is envisaged that the calibration

data from step (a) of the method according to the invention may be prepared by a manufacturer for each batch of reagents and presented as a series of standard kinetic curves. These curves would then be supplied to the customer via convenient means for storing machine readable encoded data such as software, bar codes or magnetic strips for each batch of reagents. Thus, for example, on running unknown samples, the appropriate instrument would carry the calibration curves in its software and use them as "look up tables" in order to calculate the dose of analyte in the sample under test.

Thus in a further aspect the present invention provides a method of calibrating an assay system comprising step (a) as hereinbefore defined and optionally thereafter fitting the (P_x, C_x) data to a standard equation (appropriate to time t_x). A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_x, t_x, C_x as hereinbefore defined and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown analyte forms a further aspect of the invention.

One technological area which has undergone significant advancement in recent years is the so-called point-of-care assay systems. These rely on very accurate, sensitive and rapid methods of assay to enable successful near patient testing to be performed. Clearly therefore the present invention, with the advantages referred to hereinbefore, lends itself to such technology.

The method of the invention is particularly applicable to assays of antigens or antibodies, i.e. to immunoassays, and in one embodiment of the invention the ligand under assay is an antigen and the specific binding partner comprises an antibody to the said antigen. However, as mentioned above, the invention is not to be taken as limited to assays of antibodies or antigens. Examples of ligands which may be assayed by

Claims

1. A method of assay in which a component becomes at least partly bound to a solid body characterised in that an analyte dependent parameter associated with said component is measured in a direct and continuous manner and in that said measured analyte dependent parameter is manipulated to quantitatively determine an unknown sample.

2. A method as claimed in claim 1 wherein said solid body is an optical waveguide.

3. A method as claimed in either claim 1 or claim 2 wherein said analyte dependent parameter is an optical parameter.

4. A method as claimed in any of claims 1 to 3 wherein said optical parameter is fluorescence emission.

5. A method as claimed in any preceding claim wherein said solid body is in the form of a sample containment device.

6. A method as claimed in claim 5 wherein said device is a capillary fill device.

7. A method as claimed in any preceding claim comprising the steps of

(a) calibrating the assay system for x samples each of known analyte concentration (C_x) by measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x),

(b) for an analyte of unknown concentration (C_n) measuring continuously n independent values of an analyte-dependent parameter (P_n) each at time t_n after the onset of incubation,

(c) combining the data (P_n, t_n) from step (b) with the calibration data (P_x, t_x, C_x) from step (a) to calculate the unknown dose of analyte (C_n) at time t_n.

8. Use of kinetic measurement to determine quantitatively an unknown sample in an assay system in which a component thereof becomes at least partly bound to the surface of a solid body.

9. Use as claimed in claim 8 wherein said solid body is an optical waveguide.

10. Use as claimed in either of claims 8 or 9 wherein said solid body is in the form of a sample containment device.

11. Use as claimed in claim 10 wherein said device is a capillary fill device.

12. A method of calibrating an assay system for x samples each of known analyte concentration (C_x) comprising:

(a) measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x); and, optionally

(b) fitting the calibration data to a standard equation.

13. A method as claimed in claim 12 further comprising the step of storing said calibration data on a means for storing machine readable encoded data.

14. A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_x, C_x, t_x as defined in claim 7 and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown

analyte.

15. A kit as claimed in claim 14 characterised in that the data storing means comprises a bar code marked on the device.

INTERNATIONAL SEARCH REPORT

Inte.. .tional Application No
PCT/GB 97/01248

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed byclassification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 184 600 A (BATTELLE) 18 June 1986 see abstract see page 15, line 18 - line 37 see page 16, line 22 - page 17, line 13 see page 19, line 14 - page 20, line 16 see page 25, line 10 - line 33	1-5, 7-10,12
Y	see figures 4-6,9	6,11, 13-15

Y	WO 90 14590 A (ARES-SERONO) 29 November 1990 cited in the application see abstract	6,11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 5 120 662 A (CHAN) 9 June 1992 see abstract see column 7, line 41 - line 57 see figure 11	13-15
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Int. ational Application No
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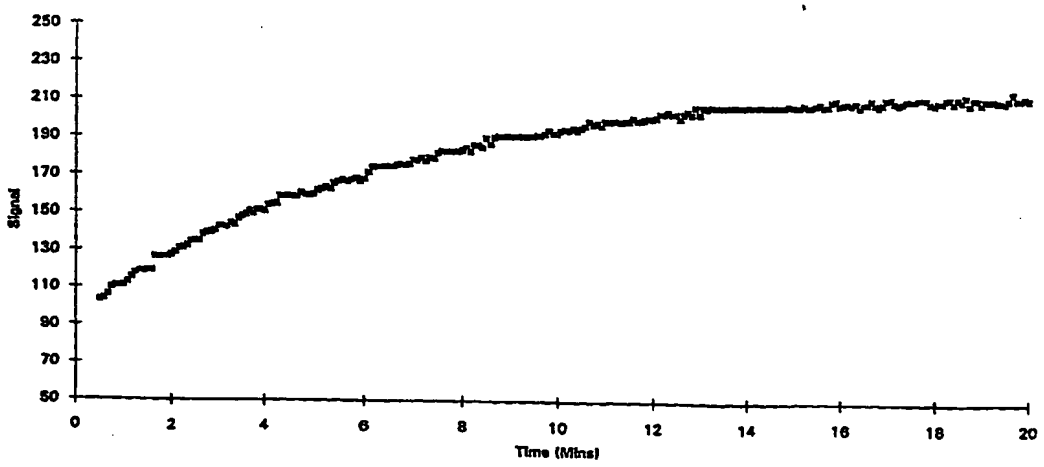

(57) Abstract

A kinetic assay method for quantifying an analyte in a sample.

Method of Assay

The present invention relates to a method of assay for quantifying an analyte in a sample, in particular to a kinetic assay method during the course of which a component of the assay system becomes at least partly bound, directly or indirectly, to the surface of a solid body.

The method of the present invention has particular applicability in the field of immunoassays and will be described herein with particular reference to immunoassays; however the method can also be used in other assays which rely on the affinity between the species to be assayed (hereinafter "ligand") and a specific binding partner for the ligand (hereinafter "specific binding partner").

The ligand concentration in such systems may be determined by monitoring the extent of complex formation or rate at which complex formation occurs. One preferred way of achieving this is by conjugating an additional component having a measurable parameter to either the ligand or its specific binding partner. This additional component is known in the art as a label and various chemical and biochemical labels are known eg. radioisotopic or biochemiluminescent species, spin labels, fluorophores, chromophores, etc. In the course of such assays, the label in effect becomes bound, indirectly at least, to a solid surface.

Conventionally, most immunoassay systems of the type described above have relied upon there being washing and/or separation step(s) in the assay protocol in order to separate bound label from label remaining in solution; the latter otherwise would be free to interfere with the bound label and lead to inaccurate results. Once separation has been effected, a variety of known techniques may be used to quantify the bound

label and thereby yield a measure of the concentration of ligand present in the sample under investigation. In such systems, the separation procedure must be repeated at each time (t) at which it is desired to make a measurement, rendering the method as a whole somewhat labour intensive and slow. In addition to these problems, there is a degree of arbitrariness in the estimation of the commencement of incubation of the assay which leads to errors in the overall timing.

In order to speed up reading the assay and/or to increase the sensitivity of conventional assay systems, it would be desirable to make kinetic measurements. The limitations referred to above mean that conventional assay systems do not lend themselves to making reliable kinetic measurements and it has been done in only a very few cases where the characteristics of the assay system allow. For example, it is known to make kinetic measurements in immunoassay systems in which enzymes are the label of choice, the rate of evolution of the product of the enzyme catalysed reaction being the parameter which is measured. In this case, measurement of the enzyme label only shortens the duration of the signal generation step and has no impact on the time taken to measure antibody:antigen binding i.e. the kinetic measurement applies to the final step of the assay and not to the key immune reaction.

Kinetic measurements have also been used in certain immunosensors. Here the rate of change of signal of the sample containing an unknown quantity of antigen is measured and compared with the same parameter for standards containing known concentration of antigen. The most convenient way of achieving this is to construct a curve of rate of change of signal (dI/dt in measurands per unit time) versus known concentration of antigen in the standard. In this way, dI/dt for the sample of interest may simply be read off the standard curve to arrive at the unknown antigen concentration. Naturally, such a technique suffers the drawback that

the assay must be allowed to attain an arbitrarily determined equilibrium at which point a single end-point measurement of the signal is made. The speed with which equilibrium is reached may be prohibitively slow and this in itself can introduce errors in the measured rate of change of signal which will be critically dependent on the prevailing conditions (eg. temperature, viscosity). Clearly it is not possible in such a system to obtain quick and accurate measurements of the ligand concentration.

Kinetic measurements have also been used to determine the concentration of an unknown in some immunoassays, as disclosed in EP-A-667528 (Daikin Industries, Limited). However, such assays do not involve the continuous monitoring of the concentration of the unknown.

In other assays systems, for example that disclosed in EP-A-184600 (Battelle Memorial Institute) kinetic measurements may be made, but are not used to determine the concentration of an unknown in a sample, with instead a single final measurement being used in this regard.

The present invention is based on the finding that, in assay methods, during the course of which a component of the assay system becomes directly or indirectly bound to, or adsorbed on, the surface of a solid body, a reliable measurement of said bound or adsorbed component (ie. without interference from the free component in solution) can be obtained by direct and continuous monitoring of said component.

It should be emphasised that the method of the invention relates to assay systems of both the direct and indirect variety, the only requirement being that they involve the binding of a component of the assay system to the surface of a solid body. Direct assay methods may typically involve monitoring the reflected and/or generated signal within an irradiated solid optical structure (eg. a waveguide) in order to

determine the extent to which (or the rate at which) the optical characteristics of said optical structure and/or the generated signal are altered by the biochemical complexation of a ligand and specific binding partner which is bound to said optical structure (eg. antigen/antibody complexation). Indirect assay methods may typically involve monitoring a label (eg. a fluorophore) bound to one or more of the components present in the assay and directly or indirectly to the solid body. Such methods are described for example in

the assay must be allowed to attain an arbitrarily determined equilibrium at which point a single end-point measurement of the signal is made. The speed with which equilibrium is reached may be prohibitively slow and this in itself can introduce errors in the measured rate of change of signal which will be critically dependent on the prevailing conditions (eg. temperature, viscosity). Clearly it is not possible in such a system to obtain quick and accurate measurements of the ligand concentration.

The present invention is based on the finding that, in assay methods, during the course of which a component of the assay system becomes directly or indirectly bound to, or adsorbed on, the surface of a solid body, a reliable measurement of said bound or adsorbed component (ie. without interference from the free component in solution) can be obtained by direct and continuous monitoring of said component.

It should be emphasised that the method of the invention relates to assay systems of both the direct and indirect variety, the only requirement being that they involve the binding of a component of the assay system to the surface of a solid body. Direct assay methods may typically involve monitoring the reflected and/or generated signal within an irradiated solid optical structure (eg. a waveguide) in order to determine the extent to which (or the rate at which) the optical characteristics of said optical structure and/or the generated signal are altered by the biochemical complexation of a ligand and specific binding partner which is bound to said optical structure (eg. antigen/antibody complexation). Indirect assay methods may typically involve monitoring a label (eg. a fluorophore) bound to one or more of the components present in the assay and directly or indirectly to the solid body. Such methods are described for example in

inter alia WO-A-88/07202 and WO-A-90/01166 (Ares Serono). The invention is equally applicable to displacement assays where the labelled component is removed from the solid surface as a result of the antibody:antigen interaction.

The novel assay method of the present invention has the advantage that an indication of the unknown ligand concentration may be obtained at a very early stage of the incubation period without the need to wait for some arbitrarily determined end-point such as equilibrium. Moreover, the operator is able to observe the result continuously and judge whether it would be worthwhile taking further readings in an attempt to improve the accuracy of the result. Additionally, continuous monitoring allows random errors caused by, for example, problems with instrumentation to be readily identified. Any spurious result may simply be isolated and ignored.

Thus in its broadest aspect the present invention provides the use of kinetic measurements to determine quantitatively an unknown sample in an assay system in which a component thereof becomes at least partly bound directly or indirectly to the surface of a solid body, for example the surface of a solid optical waveguide, electrode or piezoelectric crystal.

By "kinetic measurements" are meant direct and continuous measurements of a measurable property or effect associated with said bound component (hereinafter an "analyte dependent parameter") at a time before the assay reaches a substantially steady state i.e. equilibrium.

Viewed from a further aspect the present invention provides a method of assay in which a component becomes at least partly bound, directly or indirectly, to a solid body, for example an optical waveguide, electrode or piezoelectric crystal, characterised in that an analyte-dependent parameter associated with said component at said solid body is measured in a direct and continuous manner and in that said measured analyte dependent parameters are manipulated to quantitatively

determine an unknown sample. In one embodiment the analyte dependent parameter is an analyte dependent optical parameter (i.e. a measurable optical property or effect) but parameters relating to electrochemical or piezoelectric properties/effects may be used.

In a further embodiment, the use or method according to the invention may be applied to an analyte of known concentration for the purposes of calibration.

In a particular preferred embodiment, said method comprises the steps of:

(a) calibrating the assay system for x samples each of known analyte concentration (C_a) by measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z),

(b) for an analyte of unknown concentration (C_b) measuring continuously n independent values of an analyte-dependent parameter (P_d) each at time t_e after the onset of incubation,

(c) combining the data (P_d, t_e) from step (b) with the calibration data (P_z, t_y, C_a) from step (a) to calculate the unknown dose of analyte (C_b) at time t_e.

The analyte-dependent parameter referred to above may conveniently be any parameter associated with the interaction between applied radiation and the relevant bound assay component and includes but is not limited to light-absorbing, scattering, fluorescence emission, phosphorescence emission, luminescence emission (including chemiluminescence, bioluminescence and electrochemiluminescence) or colour emission properties. The term is also intended to encompass the measurable effects which the bound component may have for example on the refractive index or transmittability of the optical surface, on total internal reflection or surface plasmon resonance (SPR) within the solid optical body, or interactions with evanescent waves at the surface of the body. Devices and techniques for measuring such analyte dependent optical parameters or manipulating the

determine an unknown sample. In one embodiment the analyte dependent parameter is an analyte dependent optical parameter (i.e. a measurable optical property or effect) but parameters relating to electrochemical or piezoelectric properties/effects may be used.

In a further embodiment, the use or method according to the invention may be applied to an analyte of known concentration for the purposes of calibration.

In a particular preferred embodiment, said method comprises the steps of:

(a) calibrating the assay system for x samples each of known analyte concentration (C_x) by measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x),

(b) for an analyte of unknown concentration (C_n) measuring continuously n independent values of an analyte-dependent parameter (P_n) each at time t_n after the onset of incubation,

(c) combining the data (P_n, t_n) from step (b) with the calibration data (P_x, t_x, C_x) from step (a) to calculate the unknown dose of analyte (C_n) at time t_n.

The analyte-dependent parameter referred to above may conveniently be any parameter associated with the interaction between applied radiation and the relevant bound assay component and includes but is not limited to light-absorbing, scattering, fluorescence emission, phosphorescence emission, luminescence emission (including chemiluminescence, bioluminescence and electrochemiluminescence) or colour emission properties. The term is also intended to encompass the measurable effects which the bound component may have for example on the refractive index or transmittability of the optical surface, on total internal reflection or surface plasmon resonance (SPR) within the solid optical body, or interactions with evanescent waves at the surface of the body. Devices and techniques for measuring such analyte dependent optical parameters or manipulating the

above-mentioned effects are known in the art.

The invention also extends to the use of non-optical devices such as electrochemical and other sensor devices (e.g. piezoelectric crystals).

As used herein, the term "solid body" is intended to refer appropriately to any of the known surfaces to which may usefully be bound ligand and/or specific binding partner components eg. in the form of an electrochemical, optical, piezoelectric or fibre-optic biosensor as described in US-A-5356780 or an optical structure capable of exhibiting an SPR effect (eg. a diffraction grating) or a transparent optical body (eg. a prism, sheet or fibre acting as a waveguide such as is described in EP-A-170376 (Unilever)) of the type described in EP-A-171148 (Unilever) and WO-A-95/24632 (Applied Research Systems). In electrochemical assay devices where the solid body is an electrode, it is known to use components bound to magnetic beads or particles which may be attracted to a magnetic field created at the electrode. These devices too are useful in the method of the invention and are described in for example EP-A-170446 (Serono Diagnostics Limited).

In one embodiment of the invention, the solid body may be coated with a specific binding partner to the analyte of interest. Specific binding partners may be coated onto the surface of the solid body by known techniques, for example, as described in EP-A-171148.

The invention is particularly suited to assay methods during the course of which a component acting as a label and having optically measurable properties such as light absorbing, light-transmitting, light scattering, fluorescent, phosphorescent, luminescent or colour properties becomes at least partly bound (directly or indirectly) to the surface of a transparent solid body (eg. an optical waveguide), especially

methods of the type described in, for example, EP-A-170376 and EP-A-171148.

In embodiments of the invention which relate to indirect assay techniques, the binding of labels directly or indirectly to one or other of the ligand or its specific binding partner may be carried out by methods well known to the skilled man. The identity of such labels is similarly well-known to the skilled man and includes those mentioned hereinbefore.

The method according to the invention is, in certain embodiments, intended for use in specific binding assay procedures in chemical, biochemical or clinical test procedures, in particular to immunoassay procedures. Examples of such procedures are described in *inter alia* EP-A-0171148, WO-A-92/09892, WO-A-93/25892 and WO-A-93/25908.

The present method is also applicable to a wide variety of devices provided these are of a type which make use of a component bound to a solid body including, for example, dip-stick or test-strip sensors, devices using a "sample flow-through" configuration or devices employing sample containment. Sample containment devices are preferred for carrying out the method of the invention, with a more preferred device being a capillary fill device, especially a fluorescence capillary device, for example the type of device described in EP-A-171148, WO-A-90/14590 or in International patent application No. PCT/GB95/02236 (Applied Research Systems ARS Holding NV). Such capillary fill devices may be used singly or in a suitable holder such as is described in WO-A-90/1830.

In carrying out the method according to the invention to determine an unknown sample, it is first necessary to calibrate the instrument using a set of solutions containing known concentrations of analyte (ie. step (a) as defined hereinbefore). The protocol adopted for this step may be conveniently chosen by the operator and is in no way intended to restrict the scope

of the invention. Such operator determined variables include the number of standards (referred to as x above) which might typically be three or more, the number of devices, the number of readings, the time interval between readings and the total period over which calibration is carried out.

After filling a device with a particular standard, measurements of the analyte dependent parameter (referred to more generally as P_z above) are taken at regular intervals, such as every five seconds, for as long as is appropriate. This procedure is optionally repeated for further devices, yielding response data at each time point (referred to as t_y above) for all of the standard analytes. For each time point t_y it is, therefore, possible to produce a standard curve of P_z vs C_a (appropriate to time t_y). In one embodiment, the (P_z, C_a) data may be fitted to a standard equation such as an n parameter logistic equation, or appropriate algorithm, using any conventional fitting method such as a least squares method.

In step (b) of the method according to the invention, the unknown analyte-dependent parameter (referred to as P_d above) may be measured at any time-point (referred to as t_e above) and used to determine a concentration by interpolation from the standard curve (P_z, C_a) for that time point. Appropriate smoothing software may be used to improve the accuracy of the estimation of concentration of analyte in the sample. Typically the (t_e, C_b) data obtained in this step are manipulated to give a dose versus time profile for the sample, an example of which is given in Figure 2.

As has previously been emphasised, the method according to the invention is a kinetic method and in step (b) the interval between readings is operator determined and is typically of the order of less than 60s, particularly less than 30s, especially less than 10s and more especially 5s or less.

In practice, it is envisaged that the calibration

data from step (a) of the method according to the invention may be prepared by a manufacturer for each batch of reagents and presented as a series of standard kinetic curves. These curves would then be supplied to the customer via convenient means for storing machine readable encoded data such as software, bar codes or magnetic strips for each batch of reagents. Thus, for example, on running unknown samples, the appropriate instrument would carry the calibration curves in its software and use them as "look up tables" in order to calculate the dose of analyte in the sample under test.

Thus in a further aspect the present invention provides a method of calibrating an assay system comprising step (a) as hereinbefore defined and optionally thereafter fitting the (P_z, C_a) data to a standard equation (appropriate to time t_y). A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_z, t_y, C_a as hereinbefore defined and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown analyte forms a further aspect of the invention.

One technological area which has undergone significant advancement in recent years is the so-called point-of-care assay systems. These rely on very accurate, sensitive and rapid methods of assay to enable successful near patient testing to be performed. Clearly therefore the present invention, with the advantages referred to hereinbefore, lends itself to such technology.

The method of the invention is particularly applicable to assays of antigens or antibodies, i.e. to immunoassays, and in one embodiment of the invention the ligand under assay is an antigen and the specific binding partner comprises an antibody to the said antigen. However, as mentioned above, the invention is not to be taken as limited to assays of antibodies or antigens. Examples of ligands which may be assayed by

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the improved assay method of the invention are given in Table 1 below, together with an indication of a suitable specific binding partner in each instance.

Table 1

Ligand	Specific Binding Partner
antigen	specific antibody
antibody	antigen
hormone	hormone receptor
hormone receptor	hormone
polynucleotide strand	complementary polynucleotide strand
avidin	biotin
biotin	avidin
protein A	immunoglobulin
immunoglobulin	protein A
enzyme	enzyme cofactor (substrate) or inhibitor
enzyme cofactor (substrate) or inhibitor	enzyme
lectins	specific carbohydrate
specific carbohydrate of lectins	lectins

The method of the invention has very broad applicability but in particular may be used in assays for: hormones, including peptide hormones (e.g. thyroid stimulating hormone (TSH), luteinizing hormone (LH), human chorionic gonadotrophin (hCG), follicle stimulating hormone (FSH), insulin and prolactin) or non-peptide hormones (e.g. steroid hormones such as cortisol, estradiol, progesterone and testosterone, or thyroid hormones such as thyroxine (T4) and triiodothyronine), proteins (e.g. carcinoembryonic antigen (CEA) and antibodies, alphafetoprotein (AFP) and

prostate specific antigen (PSA)), drugs (e.g. digoxin, drugs of abuse), sugars, toxins, vitamins, viruses such as influenza, para-influenza, adeno-, hepatitis, respiratory and AIDS viruses, virus-like particles or microorganisms.

It will be understood that the term "antibody" used herein includes within its scope:

(a) any of the various classes or sub-classes of immunoglobulin, e.g. IgG, IgA, IgM, or IgE derived from any of the animals conventionally used, e.g. sheep, rabbits, goats or mice,

(b) monoclonal antibodies,

(c) intact molecules or "fragments" of antibodies, monoclonal or polyclonal, the fragments being those which contain the binding region of the antibody, i.e. fragments devoid of the Fc portion (e.g. Fab, Fab', $F(ab')_2$), the so-called "half-molecule" fragments obtained by reductive cleavage of the disulphide bonds connecting the heavy chain components in the intact antibody or fragments obtained by synthetic methods,

(d) antibodies produced or modified by recombinant DNA techniques, including "humanised antibodies".

The method of preparation of fragments of antibodies is well known in the art and will not be described herein.

The term "antigen" as used herein will be understood to include both permanently antigenic species (for example, proteins, peptides, bacteria, bacterial fragments, cells, cell fragments and viruses) and haptens which may be rendered antigenic under suitable conditions.

The method of the present invention is applicable to the normal range of sample types e.g. urine, serum-based and whole-blood samples, food samples such as water samples and milk samples and to the known range of assay types, for example competition or sandwich assays including *inter alia* direct antigen assays, competitive

antigen assays, direct antibody assays, sandwich antibody assays, linked antibody assays, competitive antibody assays and the like.

The detailed preparation of the assay devices within the scope of the method according to the invention and the assay procedures used to collect the data are well known to the skilled man.

The invention will now be illustrated in a non-limiting fashion by the following Examples.

EXAMPLES

Example A

1. Preparation of starting materials:

1.1 Fabrication of antibody-coated optical waveguides:

Anti-PSA monoclonal antibodies were supplied by Serono Diagnostics S A, Coinsins, Switzerland. A sheet of Permabloc glass (Pilkington Glass Ltd., St. Helens, UK) having a thickness of about 1 mm was cleaned with detergent (eg. Tween 20) in ultra-pure water with ultrasonic agitation. The surface of the glass was activated by incubating it in a 2% solution of aminopropyltrimethoxysilane in water (pH 3-4) for two hours at 75°C. After rinsing in water the glass sheet was dried at 115°C for at least four hours. The glass was then incubated for 60 minutes in a 2.5% solution of glutaraldehyde in a 0.05M phosphate buffer (pH 7) and then washed thoroughly with distilled water. Anti-PSA antibody was patterned onto the glass by discretely dosing a 1% solution of the antibody in phosphate buffer (pH 7) onto the glass and incubating it for 2 to 4 hours after which the glass sheet was washed with buffer solution. Unwanted adsorbed protein was removed by soaking with 6M urea solution in a known manner. Finally a layer of sucrose/lactose was formed over the surface of the glass sheet by spin coating. This formed plate 4 of the FCFD test device.

1.2 Preparation of PSA conjugated to allophycocyanin (APC):

A second anti-PSA monoclonal antibody, which recognises a different epitope on the PSA molecule to the one used in 1.1 above, was conjugated to allophycocyanin (λex = 650 nm, λem = 660 nm) by Molecular Probes Inc., Eugene, Oregon, USA and was used as supplied.

1.3 Microdosing of the specific reagents over a discrete zone of anti-PSA antibody:

An opaque coating was screen printed onto a clean sheet of Permabloc glass as described in GB 8911462.3. The measurement zone of the device was fabricated by microdosing a layer of anti-PSA/allophycocyanin antibody conjugate in buffer containing polyvinyl alcohol in an area 3 x 7 mm onto the glass over the zone. After the conjugate was air dried a layer of polyvinyl alcohol (4% in buffer) was microdosed over the conjugate. Finally the whole sheet of glass was coated in a layer of sucrose/lactose by spray coating. This formed plate 2 of the FCFD test device.

1.4 Fabrication of FCFD test devices:

FCFD test devices such as have been described in EP-A-0171148 were fabricated by screen printing onto the waveguide resulting from 1.1 above bonding tracks of an ultraviolet curing glue (UVS 91, Norland Inc., USA) containing glass microspheres of 100 μm diameter (Jencons Ltd., UK) in a pattern defining the long edges of the capillary cell devices. A sheet of glass as defined in 1.3 above was then placed over the waveguide and a vacuum applied to the laminate. As a result of the vacuum the upper sheet of glass was caused to press down onto the glue, the glass microspheres defining a gap of 100 μm between the glass sheets. The laminate

was then exposed to an ultraviolet light source to cure the glue. Finally, the laminate sheet was broken into individual test devices as described in EP-A-0171148.

1.5 Apparatus used in the measurement of the PSA assay:

A simple fluorimetry apparatus comprising a continuous light source (provided by light emitting diodes which emit light at a suitable wavelength to excite the allophycocyanin fluorophore) and a photomultiplier tube (PMT). Light emerging from the optical edge of the FCFD is filtered to remove stray pump light and the discrete angle range required to read the bound fluorescence measured by focusing the light onto the PMT through an aperture.

2. Assay Procedure for PSA:

Signals indicative of analyte concentration were obtained from the FCFD devices by the following method. The device, containing the sample to be assayed, was flood illuminated with light appropriate to stimulate the fluorophore contained within the test reagentry. This input light is continuous and its intensity is repeatable at every required measurement time point.

In estimating the concentration of an unknown sample it is first necessary to calibrate the instrument using a set of solutions containing known concentrations of analyte. For the data presented here seven standard concentrations were used, each concentration being run in duplicate devices. After filling a device with a particular standard concentration, measurements of the level of fluorescence were taken at regular intervals (every 5 seconds for the data presented). In this way the kinetics of the reaction could be monitored, as demonstrated in Figure 1. Measurements were taken over a period of 20 minutes. After completing kinetic

measurements on all devices, data was available (at all of the time points) regarding the response to all the particular standard analyte concentrations. It was therefore possible to produce a "standard curve" corresponding to each of the time points. In the case of the present data the standard equation used was a four parameter logistic, having been fitted by a conventional least squares method.

Subsequent to this calibration procedure, samples of unknown concentration were run in the same kinetics mode. The fluorescence level at any time-point was interpolated off the associated standard curve enabling a concentration level to be ascertained. The results of the dependence of the measured dose on assay time interpolated from the relevant standard curve are shown in Figure 2.

Claims

1. A method of assay in which a component becomes at least partly bound to a solid body characterised in that an analyte dependent parameter associated with said component is measured in a direct and continuous manner and in that said measured analyte dependent parameter is manipulated to quantitatively determine an unknown sample and in that the results of the determination are monitored continuously.

2. A method as claimed in claim 1 wherein said solid body is an optical waveguide.

3. A method as claimed in either claim 1 or claim 2 wherein said analyte dependent parameter is an optical parameter.

4. A method as claimed in any of claims 1 to 3 wherein said optical parameter is fluorescence emission.

5. A method as claimed in any preceding claim wherein said solid body is in the form of a sample containment device.

6. A method as claimed in claim 5 wherein said device is a capillary fill device.

7. A method as claimed in any preceding claim comprising the steps of

(a) calibrating the assay system for x samples each of known analyte concentration (C_a) by measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z),

(b) for an analyte of unknown concentration (C_b) measuring continuously n independent values of an analyte-dependent parameter (P_d) each at time t_s after

the onset of incubation,

(c) combining the data (P_d, t_e) from step (b) with the calibration data (P_z, t_y, C_a) from step (a) to calculate the unknown dose of analyte (C_b) at time t_e.

8. A method of calibrating an assay system for x samples each of known analyte concentration (C_a) comprising:

(a) measuring continuously for each sample independently at a plurality of times (t_y) after the onset of incubation the value of an analyte-dependent parameter (P_z); and, optionally

(b) fitting the calibration data to a standard equation.

9. A method as claimed in claim 8 further comprising the step of storing said calibration data on a means for storing machine readable encoded data.

10. A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_z, C_a, t_y as defined in claim 7 and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown analyte.

11. A kit as claimed in claim 10 characterised in that the data storing means comprises a bar code marked on the device.

Claims

1. A method of assay in which a component becomes at least partly bound to a solid body characterised in that an analyte dependent parameter associated with said component is measured in a direct and continuous manner and in that said measured analyte dependent parameter is manipulated to quantitatively determine an unknown sample.

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3. A method as claimed in either claim 1 or claim 2 wherein said analyte dependent parameter is an optical parameter.

4. A method as claimed in any of claims 1 to 3 wherein said optical parameter is fluorescence emission.

5. A method as claimed in any preceding claim wherein said solid body is in the form of a sample containment device.

6. A method as claimed in claim 5 wherein said device is a capillary fill device.

7. A method as claimed in any preceding claim comprising the steps of

(a) calibrating the assay system for x samples each of known analyte concentration (C_x) by measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x),

(b) for an analyte of unknown concentration (C_n) measuring continuously n independent values of an analyte-dependent parameter (P_n) each at time t_n after the onset of incubation,

(c) combining the data (P_n, t_n) from step (b) with the calibration data (P_x, t_x, C_x) from step (a) to calculate the unknown dose of analyte (C_n) at time t_n.

8. Use of kinetic measurement to determine quantitatively an unknown sample in an assay system in which a component thereof becomes at least partly bound to the surface of a solid body.

9. Use as claimed in claim 8 wherein said solid body is an optical waveguide.

10. Use as claimed in either of claims 8 or 9 wherein said solid body is in the form of a sample containment device.

11. Use as claimed in claim 10 wherein said device is a capillary fill device.

12. A method of calibrating an assay system for x samples each of known analyte concentration (C_x) comprising:

(a) measuring continuously for each sample independently at a plurality of times (t_x) after the onset of incubation the value of an analyte-dependent parameter (P_x); and, optionally

(b) fitting the calibration data to a standard equation.

13. A method as claimed in claim 12 further comprising the step of storing said calibration data on a means for storing machine readable encoded data.

14. A kit comprising an assay device together with means for storing machine readable encoded data which contains calibration data P_x, C_x, t_x as defined in claim 7 and which is adapted to cooperate with reading means for the purpose of quantitatively determining an unknown

analyte.

15. A kit as claimed in claim 14 characterised in that the data storing means comprises a bar code marked on the device.

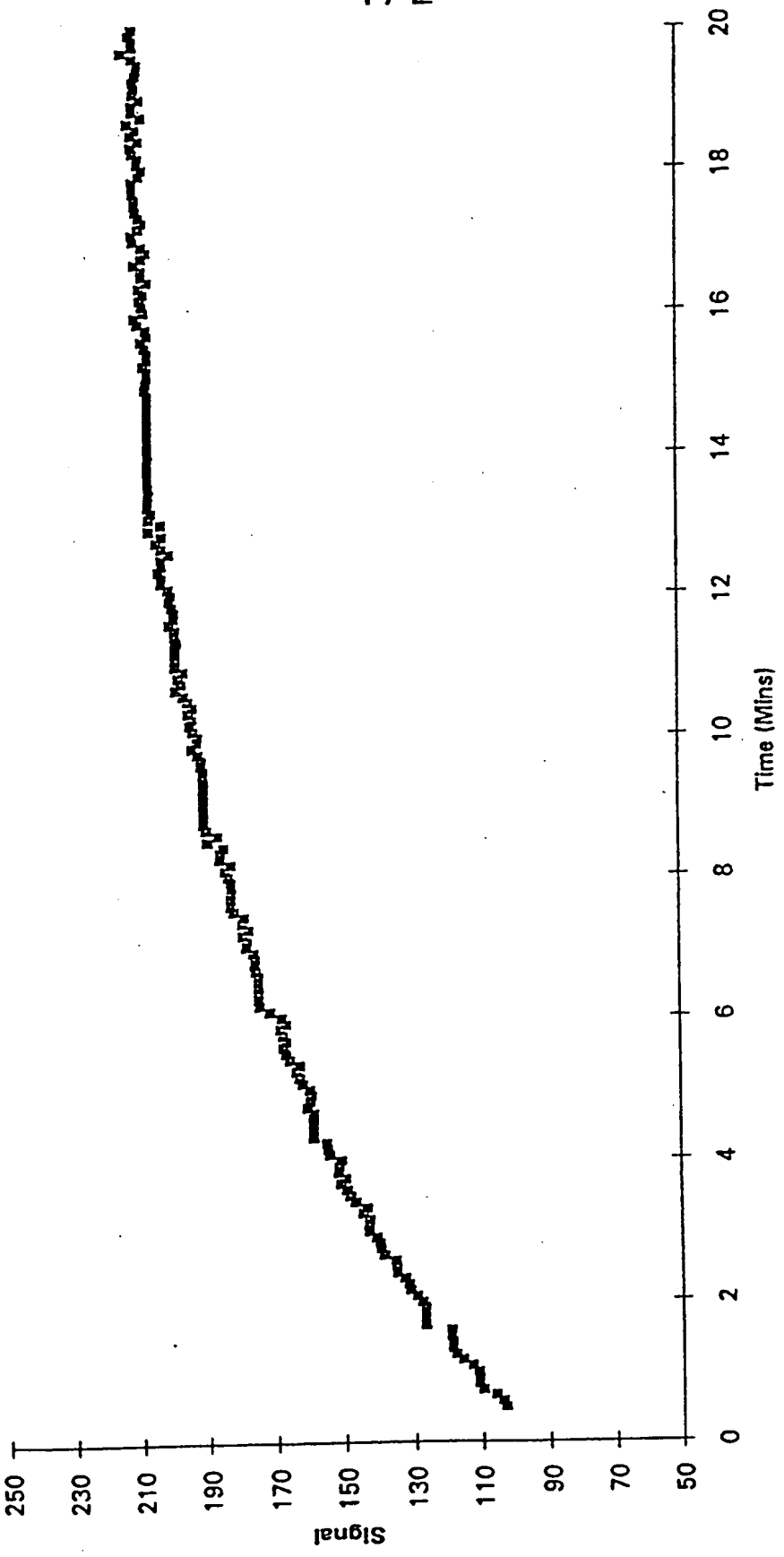

Figure 1

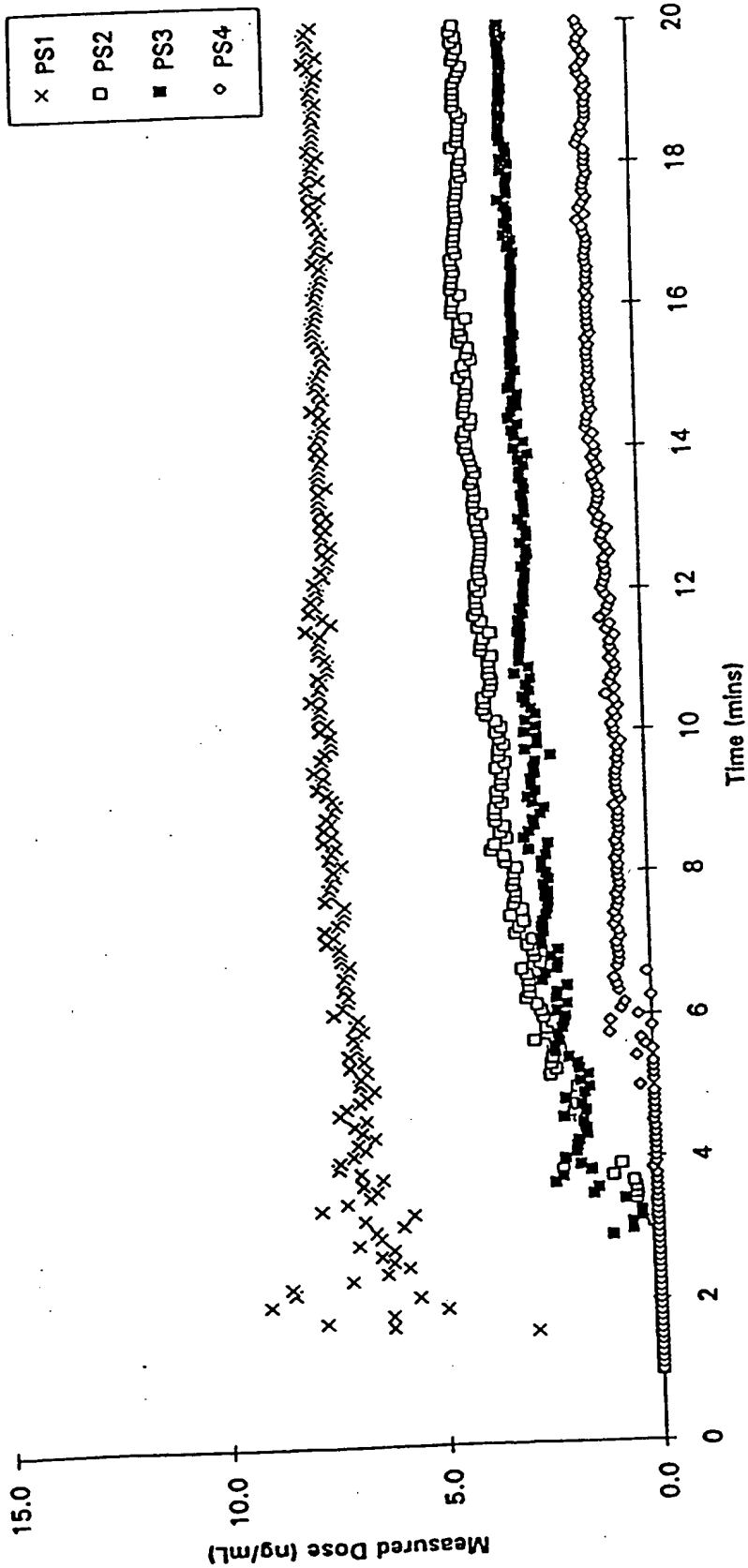
PS1
PS2
PS3
PS4
Measured Dose (ng/mL)
15.0
10.0
5.0
0.0
Time (mins)
0
2
4
6
8
10
12
14
16
18
20

Figure 2

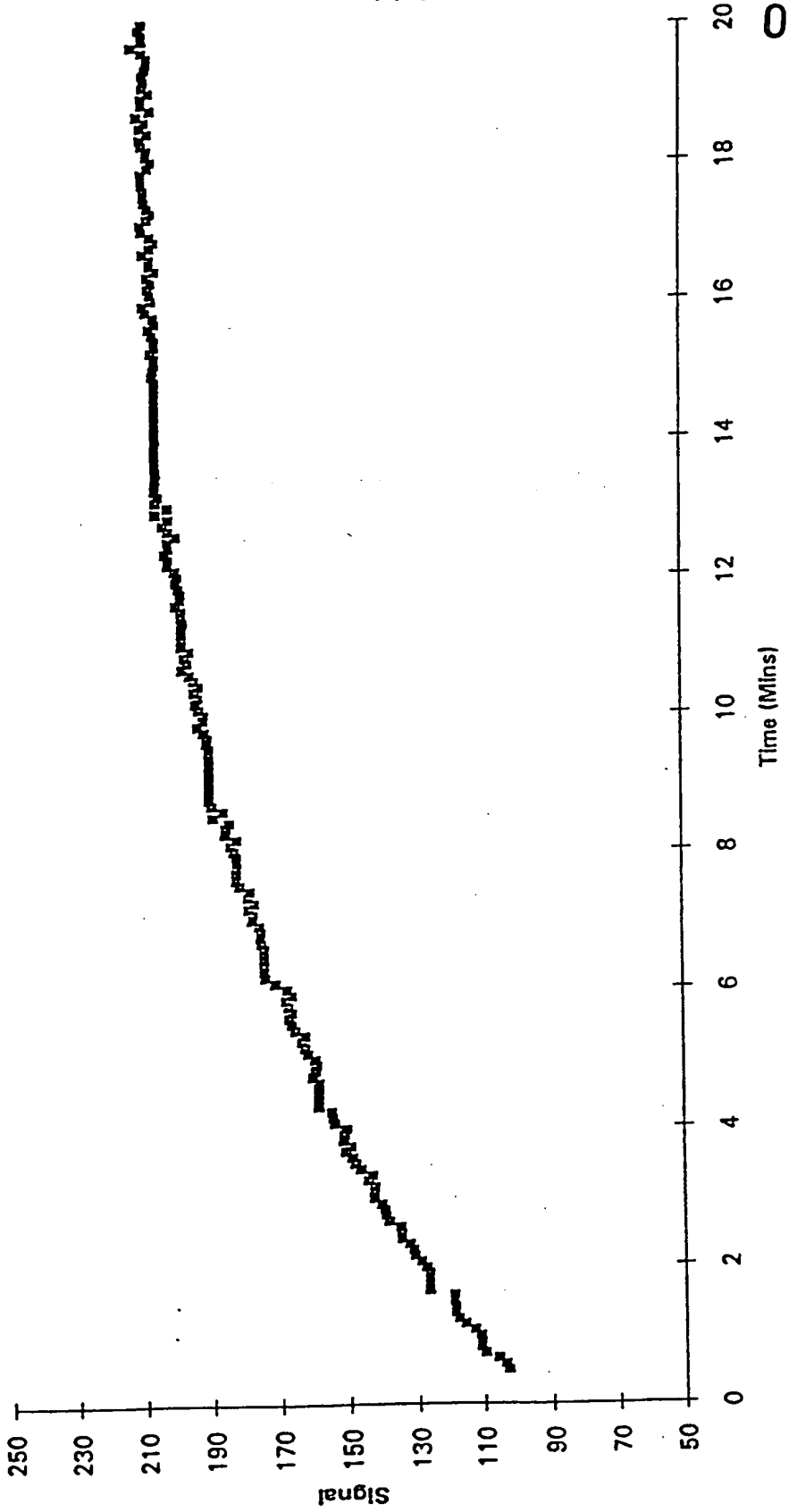

Figure 1

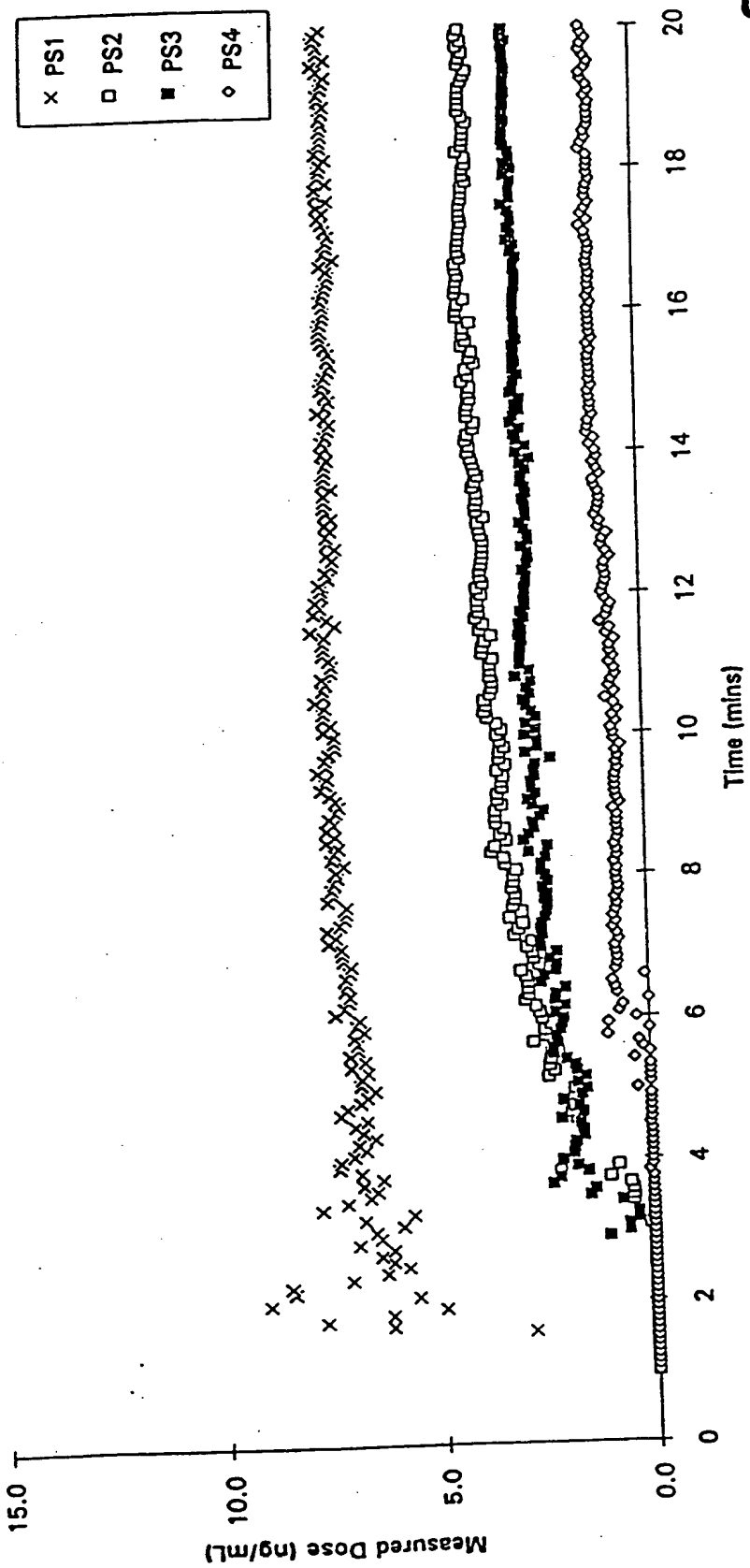
PS1
PS2
PS3
PS4
Measured Dose (ng/mL)
15.0
10.0
5.0
0.0
Time (mins)
0
2
4
6
8
10
12
14
16
18
20

Figure 2